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RESPONSE OF PACLOBUTRAZOL IN SELECTED BIOASSAYS

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SUMMARY

Present investigation was carried out to analyse the response of varying concentrations of paclobutrazol in plant tissues. Evidences were produced by examining the interaction of gibberellic acid (GA_3) and paclobutrazol (PBZ) in lettuce hypocotyl bioassay and 6-benzyl aminopurine (BAP) and paclobutrazol in radish cotyledon enlargement test that paclobutrazol counteracts gibberellin activity when applied exogenously. It also favoured radish cotyledon enlargement at low concentration without counteracting BAP action. Paclobutrazol slightly increased radish cotyledon growth at low concentration (up to 0.01 ppm) and could not counteract the growth caused by 1.0 ppm BAP at its 0.001 to 1.0 ppm concentrations suggesting independent effect of paclobutrazol to that of BAP. Furthermore, the activity of paclobutrazol was tested in corn root curvature test and it was found that PBZ concentrations from 0.001 to 10.0 ppm linearly increased the percentage of corn roots showing more than 90^0 curvature.

***Key words*: Paclobutrazol, cultar, lettuce hypocotyle growth test, radish cotyledon enlargement test, corn root curvature test**

INTRODUCTION

Paclobutrazol, a plant growth regulator and a representative of triazole compounds, has been extensively used in tropical fruits. The most useful benefits include advancing or enhancing flowering in mango (Tongumpai *et al.* 1991), durian (Chandraparnik *et al.* 1992) avocado (Obando *et al.* 1992), apple (El Hodairi *et al.* 1990), citrus (Martínez-Fuentes *et al.* 2004), litchi (Menzel and Simpson 1990) and also crops grown on trellises such as grapes (Shaltout *et al.* 1988) and kiwi fruit (Henzell *et al.* 1992). A range of other tropical fruits have responded to paclobutrazol treatment but the commercial benefits need to be quantified. Interesting new areas of development are the use of Cultar in re-juvenating old orchards, increasing juvenile bearing, alleviating biennial bearing, establishment of high density planting and interactions with other plant growth regulators.

Reduction of shoot growth by paclobutrazol is always accompanied by lowered content of gibberellin (Lever 1986). Gibberellins are synthesized from mevalonic acid via the isoprenoid pathway and the paclobutrazol specifically inhibit the microsomal oxidation of kaurene, kaurenol and kaurenal catalyzed by kaurene oxidase (Hedden and Graebe 1985). Kurian *et al.* (1992) reported that soil drenched with 2.5 g PBZ per mango tree had lower activity of zeatin, zeatin riboside, isopentenyl adenosine and isopentenyl adenine than that of control plants. Therefore, present investigation was carried out to find out the interaction of gibberellins and paclobutrazol in lettuce hypocotyl bioassay, benzyl amino purine and paclobutrazol in radish cotyledon enlargement

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tests and activity of paclobutrazol in corn root curvature test.

MATERIALS AND METHODS

The lettuce hypocotyl growth test (Frankland and Wareing 1960) was conducted employing EC-15728 seeds germinated at 24±1^{0}C in darkness for 48 hours. Seedlings with 0.5-0.6 cm long radicals were selected. In each beaker (50 ml) five seedlings were kept with 2 ml of test solutions. The test solutions comprised of different concentrations of either gibberellic acid (0.001 to 10 ppm) or paclobutrazol (0.001 to 10 ppm) alone, and various combinations of different concentrations of paclobutrazol with a constant level of gibberellic acid (1.0 ppm). The seedlings were allowed to grow under continuous light for 72 hours at 24±1^{0}C and the hypocotyl lengths were then measured.

Radish cotyledon enlargement test (Letham 1971) was conducted for the detection of cytokinin like activity. The radish seeds of cv. Pusa Chetki were germinated at 25±1^{0}C in dark for 36 hours. Afterwards the inner cotyledons were excised and uniform cotyledons were selected. These cotyledons were placed in petriplates containing Whatman No. 1 paper discs. In each petriplate, 2.5 ml water or test solutions and five cotyledons were placed. These test solutions includes BAP (0.001 to 10 ppm) or paclobutrazol (0.001 to 10 ppm) alone, and various combinations of different concentrations of paclobutrazol with a constant level of BAP (1.0 ppm). These cotyledons were allowed to grow under constant light for 72 hours and then the cotyledons were weighed. The GA_3 and BAP used in this study were procured from Sigma Chemicals, U.S.A.

Paclobutrazol activity was tested in corn root curvature test as described by Ram and Raina (1992). Five ml test solution (paclobutrazol 0.001 to 10 ppm) was poured in each petriplate containing Whatman No.1 paper discs (9.0 cm). Ten seeds of corn cv. Sweta, presoaked and thoroughly washed were arranged introversely on the periphery of paper discs. The petriplates were kept at 25±1^{0}C in dark. The number of roots having more than 90^0 curvature were counted after 72 hours.

The experiments were laid out in completely randomized block design and each treatment was replicated 5 times. Valid conclusions were drawn only on significant differences between the treatment means at 0.05 level of probability (Cochran and Cox 1950).

RESULTS AND DISCUSSION

Response of paclobutrazol in different bioassays: Different concentrations (0.001 to 10 ppm) of PBZ were tested for possible interaction with gibberellic acid and cytokinin (BAP) following lettuce hypocotyl growth test and radish cotyledon enlargement test, respectively, as well as in corn root curvature test.

Lettuce hypocotyl growth test: Linear increase in hypocotyl length was observed with the increasing concentrations of GA_3 (Fig. 1a). However, it is interesting to note that lettuce hypocotyl length was found to linearly decrease by paclobutrazol (Fig. 1b). It is known that reduction in shoot growth by paclobutrazol is accompanied by lower content of gibberellins. Reduced level of gibberellins have also been detected as a result of paclobutrazol treatment (Hedden and Graebe 1985, Lever 1986). Influence of paclobutrazol both on gibberellic acid and phytosterol formation affected the longitudinal shoot growth in the regulation of cell elongation which appeared closely linked to the availability of gibberellins. The physiological reason for this effect was probably an altered sterol synthesis and change in membrane property (Dennis and Nitsch 1966, Haughan *et al.* 1987). In cases where paclobutrazol was applied in different concentrations with a constant level of GA_3 (1.0 ppm), paclobutrazol in concentrations of 0.01 ppm

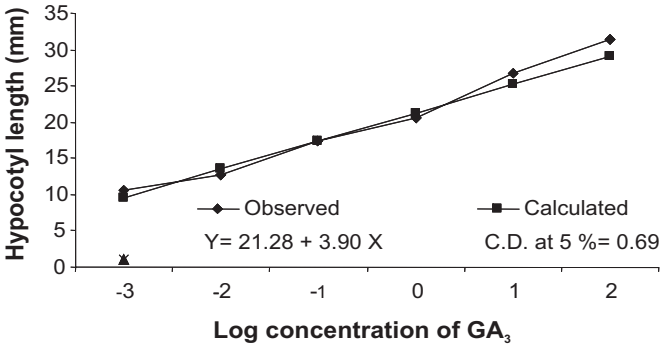

Fig. 1a. Response of lettuce hypocotyl growth test for GA

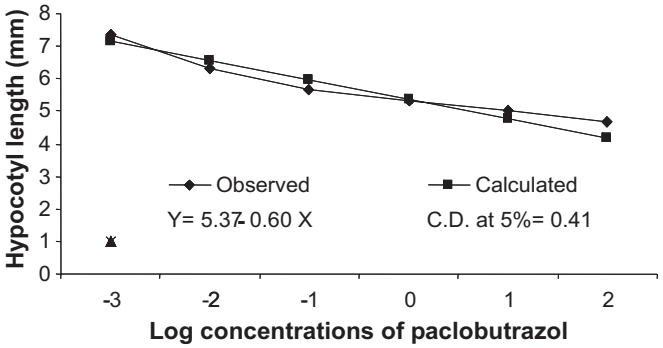

Fig. 1b. Response of lettuce hypocotyl growth test to paclobutrazol

to 10.00 ppm counteracted linearly the elongation of lettuce hypocotyls caused by GA_3 (Fig 1c). Thus, paclobutrazol not only inhibited growth of lettuce hypocotyl but also counteracted the growth promotion by GA_3 in lettuce hypocotyl bioassay, suggesting counteraction of the growth caused by endogenous gibberellin or gibberellin applied exogenously.

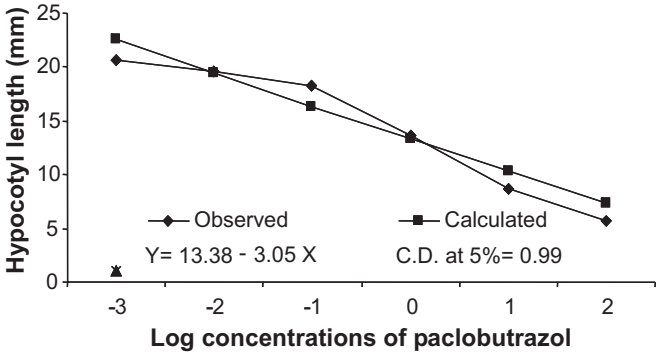

Fig. 1c. Response of lettuce hypocotyl growth test to PBZ+GA_3 (1.0 ppm)

Radish cotyledon enlargement test: Data in Fig. 2a showed that 0.01 to 10.0 ppm BAP increased growth of radish cotyledons. However, paclobutrazol increased the growth of radish cotyledon at 0.01 ppm but decreased when applied at 0.1 to 10.0 ppm in the medium (Fig. 2b). Interestingly, paclobutrazol could not counteract the growth caused by 1.0 ppm BAP. Paclobutrazol decreased cotyledon growth beyond 1.0 ppm (Fig. 2c). Thus paclobutrazol slightly increased radish cotyledon growth at low concentration (up to 0.01 ppm) but could not counteract the growth caused by 1.0 ppm BAP at 0.001 to 1.0 ppm concentrations suggesting independent effect of paclobutrazol to BAP. Kurian *et al.* (1992) reported that soil drenched with 2.5 g PBZ per mango tree had

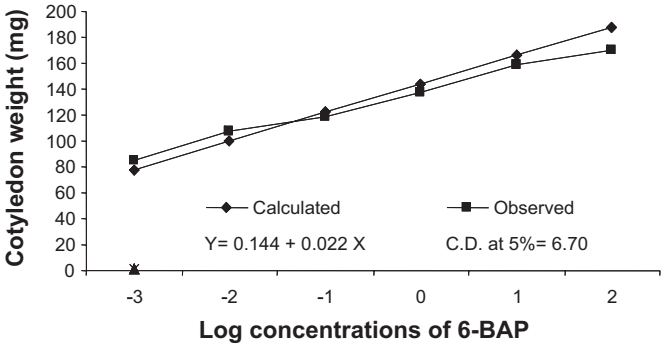

Fig. 2a. Response of radish cotyledon enlargement test for 6-BAP

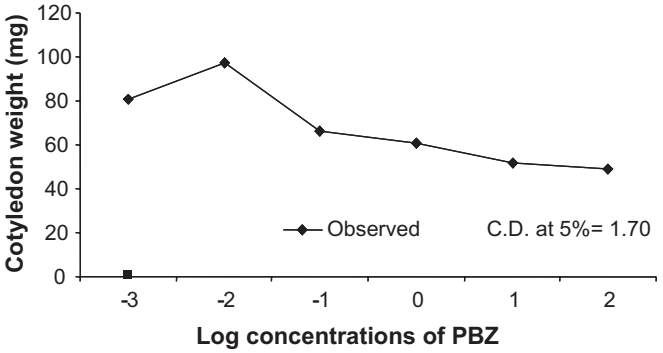

Fig. 2b. Response of radish cotyledon enlargement test for PBZ

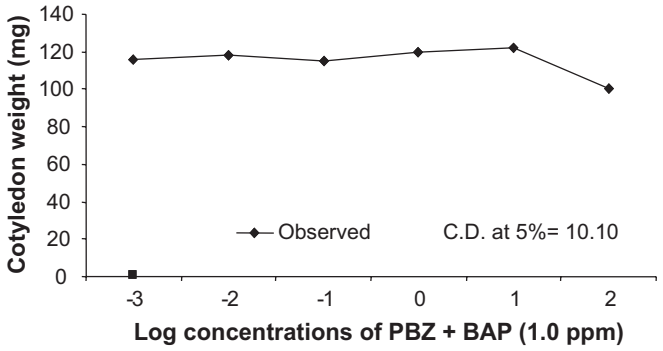

Fig. 2c. Response of radish cotyledon enlargement test for PBZ+BAP (1.0 ppm)

lower activities of cytokinin like substances than that in control trees. However, paclobutrazol increased zeatin and zeatin riboside content in raspberry (Kur'yata *et al.* 1991)

Corn root curvature test to paclobutrazol: Paclobutrazol concentrations from 0.001 to 10.0 ppm increased the percentage of corn roots showing more than 90^0 curvatures (Fig. 3). This increase was linear with the increase in concentrations of paclobutrazol. On

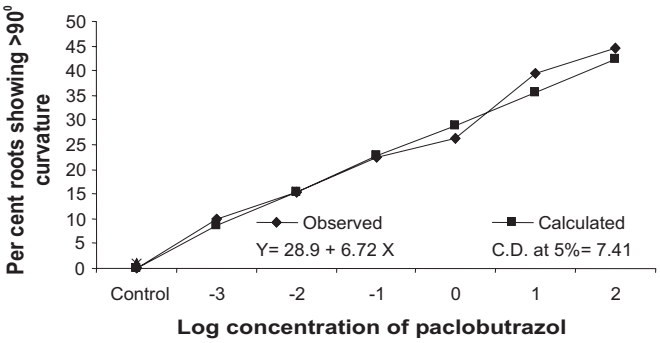

Fig. 3. Response of corn root curvature (>90⁰) for paclobutrazol

the basis of observed and calculated frequencies of roots showing >90⁰ curvature, the equation Y=28.9 + 6.72 X was derived.

Results suggest that paclobutrazol counteracts activity induced by gibberellic acid when applied exogenously. It also favoured radish cotyledon enlargement at low concentration without counteracting the action of BAP. Furthermore, paclobutrazol linearly increased the frequency of corn roots showing >90⁰ curvature.

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